"Use of Polymerase Chain Reaction (PCR) and Spore Trapping for Efficient Fungicide Management of Asian Rust in Soybeans"

Final Report

Contract Number: 20008
Amount of Funding Awarded: $24,800.00
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Report Submitted on: April 18, 2006

This project was initiated over the concerns held by producers and consultants involved in the production of soybeans about the risks posed by Asian rust, Phakospora pachyrhizi. These concerns involved whether real dangers to productivity might occur in the face of this disease, and what means could be employed to manage it in a cost effective manner. The main management tool against rust is timely applications of protectant fungicides. These will occur in response to influxes of spores transported by weather fronts and deposited in rain from infected soybeans acres to the south. Currently producers must stay informed about what is happening to the south and rely on sentinel plots of early planted beans as indicators of rust influx .It is our goal to provide an early warning system of these influxes using PCR technology and to provide producers and consultants with information leading to the efficient use of chemical protectants.

In many years rust may fail to move northward early enough to justify the need for protectants, however, the development of rust infections in states to our south and west may cause unwarranted applications to made as insurance against disease. In other years, early influx of spores will warrant the application of protectants especially if disease conducive weather is forecasted. No system currently exists for giving producers and consultants enough early warning to get these protectants on in a timely fashion should a spore influx occur. Over 1.5 million acres of soybeans are grown in the state. Unwarranted treatments with protectants over these acres will represent millions in lost net revenue to producers. Un-timely treatments or unsprayed acres in times of need will represent millions in lost net revenue due to yield loss associated with disease.

This project represents a collaboration with producers, crop consultants and UW extension to test an early warning system for Asian rust of soybeans using cutting edge technology for real time detection of spore influxes by rain. The involvement of independent crop consultants, whose management expertise and knowledge of the specific environment on individual farms is a key component to adopting this technology for practical application. Eventually a grower organization funded spore trapping network could provide forecasting for all growers for pennies per acre cost, otherwise, only the larger producers or crop consulting firms will be able to adopt this technology.
Our main objectives in this project were:

1. Develop a cheap and effective rain based spore trap.

2. Organize a network of independent crop consultants to maintain the traps and monitor soybean fields in the vicinity, with associated fungicide application trials to assess spray timing efficacy in the event of disease development.

3. Develop the real-time PCR methodology for Asian rust spore detection from trap samples, and test our methods by monitoring a commonly occurring indicator species of rust (Puccinia coronata) known to be deposited by rain.

4. Utilize the UW extension specialists in soybean plant pathology to provide quality control on our use of the PCR technology.

We were successful to a very high degree at accomplishing our objectives in 1 - 3. The traps at a cost of $15.00 each, performed well and were easy for the consultants to maintain. We received trap filters by mail within 1 - 2 days of sending. We did not achieve a rapid turn around for test results in 2005, however a system is now in place to provide 48 hour turn around. The consultants provided suggestions for trap improvements for 2006 involving a better system for holding down the filters in the trap, and exclusion of critters. These improvements have already been adopted. In addition we plan to change the filters once per week regardless of rain to ensure cleaner filter samples when rain occurs. The trap was effective without these improvements at trapping the indicator species, Puccinia coronata, and our PCR methods were effective at its detection. Our objective of using the soybean plant pathology specialists at UW-Madison to confirm our results has not been fruitful. Following repeated enquiries to obtain confirmation of our results on 30 samples for Puccinia coronata submitted 11/9/05, no such results have been provided. Subsequently we submitted 10 filter samples collected during the season indicating the possible presence of Asian rust to Dr Les Szabo, USDA, St. Paul, MN. His testing confirmed the presence of Asian rust in one of the samples where we produced a higher level positive test for Asian rust.

It is clear that the infrastructure and technology exists to provide an early warning of Asian rust spore influx to assist producers in decisions regarding the application of protectants. Based on the trapping results, consultant field monitoring, fungicide trials and prevailing environment in 2005, no commercial fields involved in our network were treated for rust, or the treat of rust. Insurance treatments were avoided as planned with out risk to the producer. No disease developed anywhere in the state, even though it is now known that rust spore influxes did occur during the 2005 growing season. Had more disease conducive weather prevailed in 2005 only ourselves and Dr. Les Szabo would have been aware of the possible risk for disease development. A packet of PCR data for some of the tests has been provided to illustrate examples of our positive results in the detection of Asian rust, Phakospora pachyrhizi and Puccinia coronata using PCR and rain trap samples.

The project will require a few more years of testing to confirm whether such a system will provide an effective means of timing protectant applications in years conducive to disease development. We plan to make the following improvements in 2006:

1. Incorporate the more sensitive TAQMAN probe technology into the PCR detection, allowing for more precise quantification of spore influx load.

2. Locate cooperators in the spore trapping network for more uniform coverage of the soybean acres in the state, and work with UW extension specialists to fill in any gaps in coverage.

3. Enlist a number of cooperators in Iowa and Illinois to monitor traps as a possible early warning of Asian rust spore influx into areas downwind from Wisconsin.

4. Eliminate fungicide trials as part of the project and increase the number of fields scouted in the vicinity of each spore trap. Fungicide applications made to any project field will still be documented.

The incorporating of aerobiological models which predict the risk of disease establishment based on weather, is a long term goal of this project and our data generated should confirm whether forecasting models, or spore trapping, provides the most cost effective means for protectant application decisions. Either way such a system is dependent on input from highly trained and motivated crop consultants who can evaluate the situation on the ground in the interest of the producer. Ultimately any system used must provide dependable information at a reasonable cost to the producer to help them manage the risk associated with Asian rust of soybean. The current system proposed of using sentinel plots of early planted soybeans as an indicator of spore influx can only be considered temporary until a true forecasting system can be established.

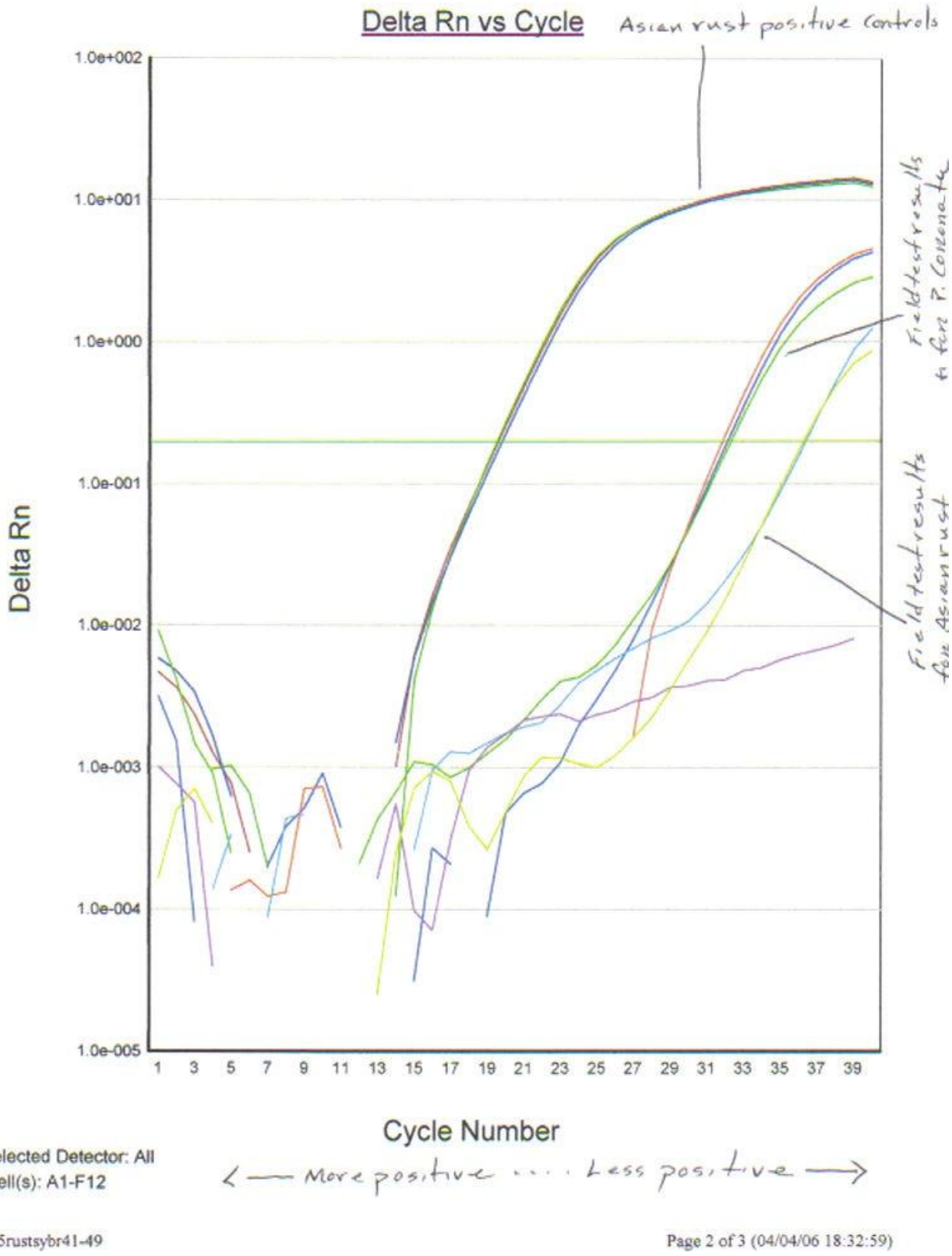
File:	715rustsybr41-49
Print Date:	Tuesday, April 04, 2006 18:32:59
User:	Linda Kotolski
Plate Type:	Absolute Quantification
PCR Volume:	50 µL

Document Information

Operator:	Linda Kotolski
Run Date:	Friday, July 15, 2005 10:34:21
Last Modified:	Friday, July 15, 2005 15:45:48
Instrument Type:	Applied Biosystems 7300 Real-Time PCR System

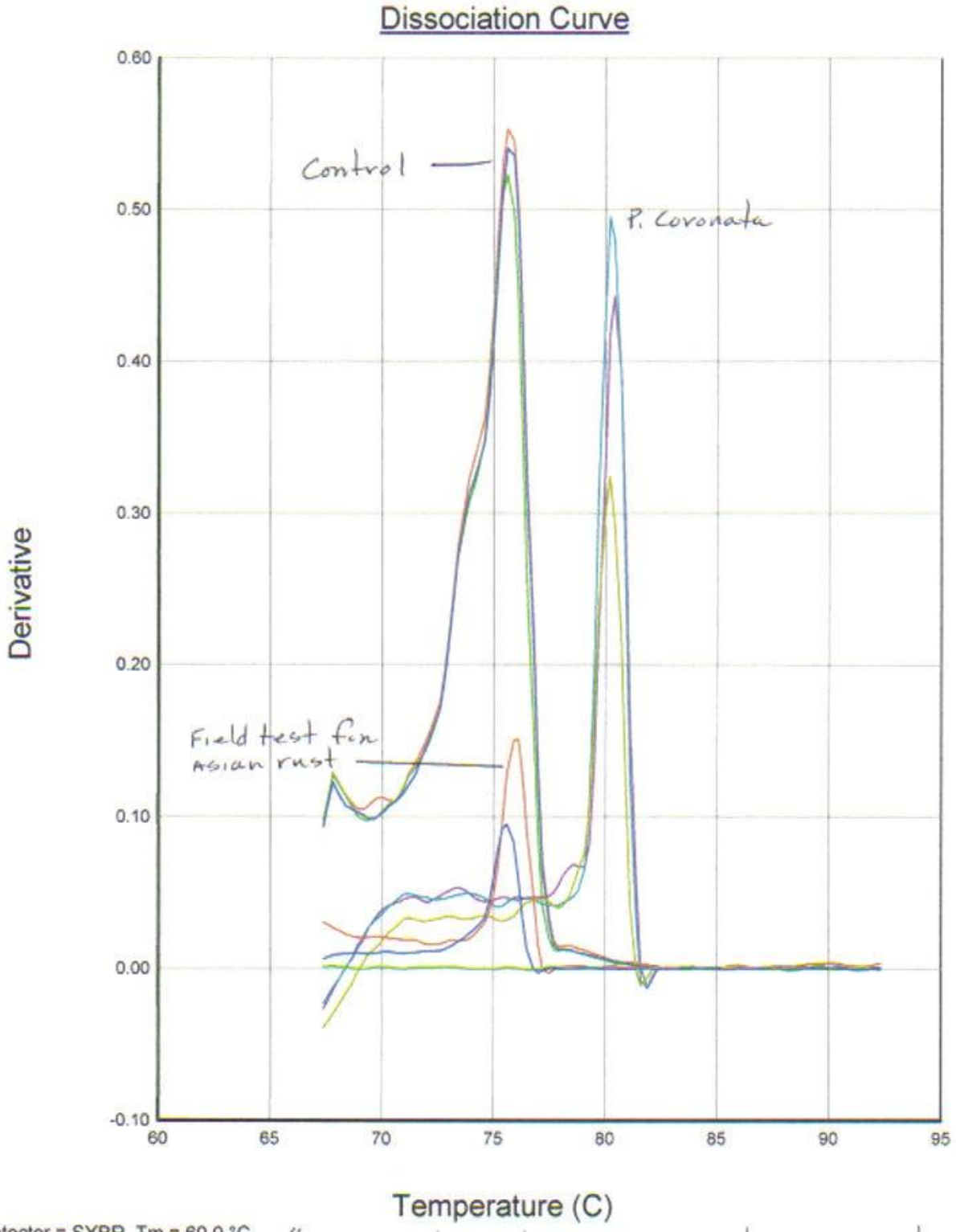
Well	Sample Name	Ct	StdDev Ct	Qty	Tm
A4	ppa1	19.52	0.154	1.00	75.6 °C
A5	ppa1	19.45	0.154	1.00	75.6 °C
A6	ppa1	19.75	0.154	1.00	75.6 °C
D7	cor41	32.09	0.220	3.32e+012	80.4 °C
D8	cor41	31.82	0.220	1.76e+012	80.2 °C
D9	cor41	32.25	0.220	4.79e+012	80.2 °C
F10	ppa41	36.32	0.071	5.52e+016	76.1 °C
F11	ppa41	Undet.	0.071		69.9 °C
F12	ppa41	36.22	0.071	4.39e+016	75.6 °C

4



715rustsybr41-49

Page 2 of 3 (04/04/06 18:32:59)



Detector = SYBR, Tm = 60.0 °C
Well(s): A1-F12

"Dissociation at same temperature indicates presence of pathogen"

715rustsybr41-49

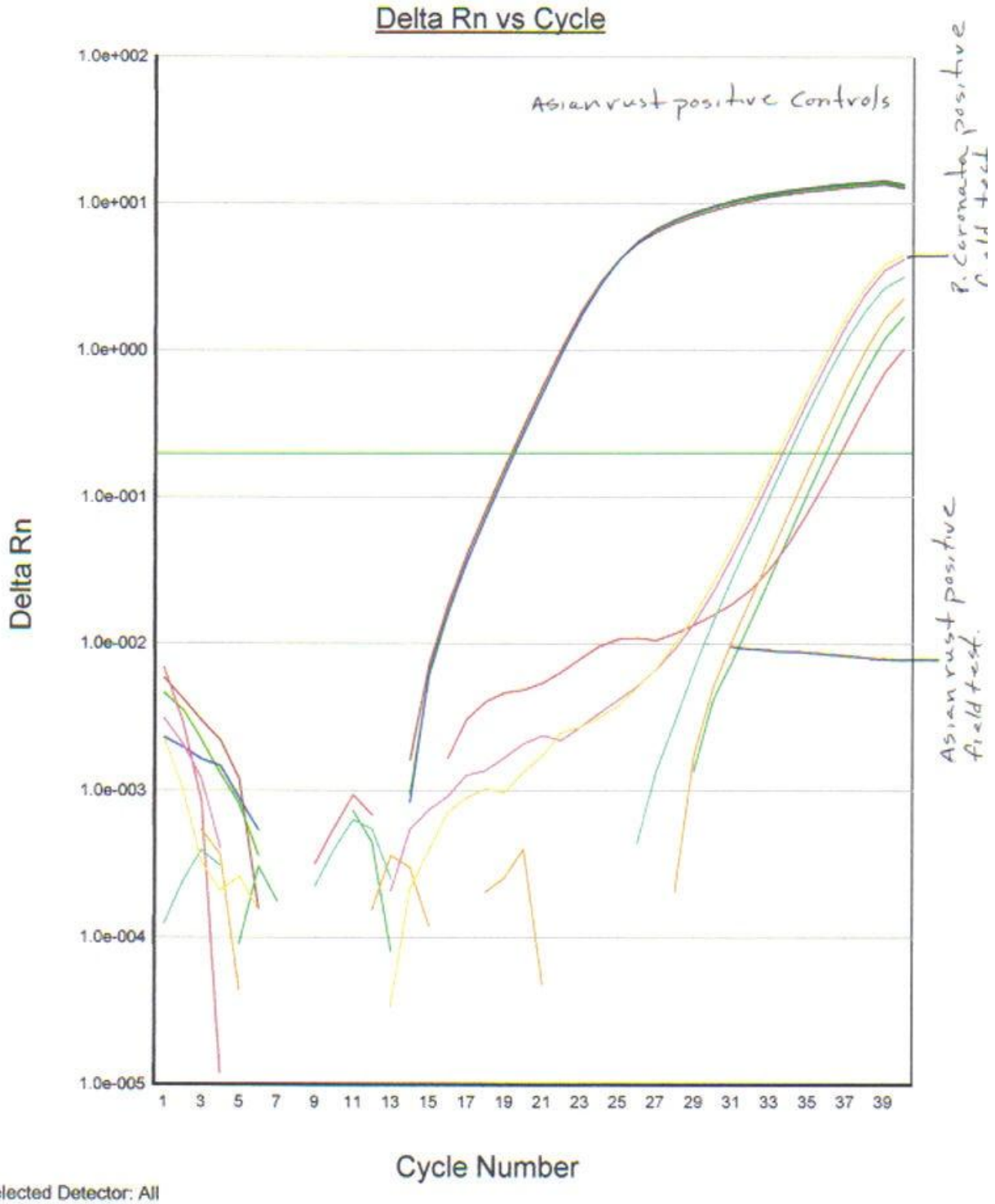
Page 3 of 3 (04/04/06 18:32:59)

File:	721rust27-37
Print Date:	Tuesday, April 04, 2006 18:24:08
User:	Linda Kotolski
Plate Type:	Absolute Quantification
PCR Volume:	50 µL

Document Information

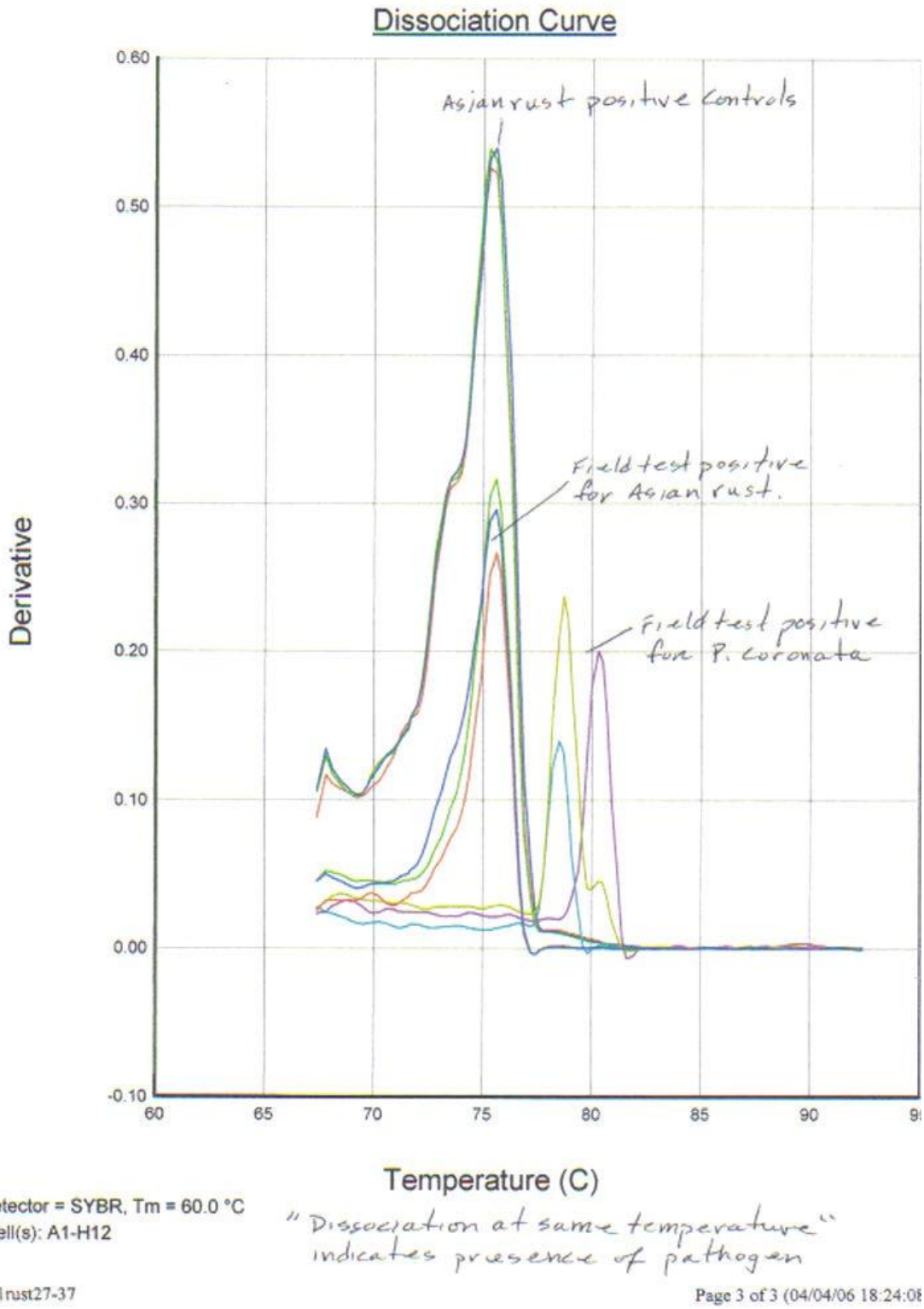
Operator:	Linda Kotolski
Run Date:	Thursday, July 21, 2005 07:47:26
Last Modified:	Thursday, July 21, 2005 10:14:52
Instrument Type:	Applied Biosystems 7300 Real-Time PCR System

Well	Sample Name	Ct	StdDev Ct	Qty	Tm
A4	ppal ng	19.26	0.080	1.00	75.3 °C
A5	ppal ng	19.39	0.080	1.00	75.3 °C
A6	ppal ng	19.40	0.080	1.00	75.6 °C
E10	34cor	35.91	0.656	3.62e+016	80.3 °C
E11	34cor	36.64	0.656	1.97e+017	78.5 °C
E12	34cor	35.33	0.656	9.64e+015	78.7 °C
H1	34ppa	34.00	0.333	4.48e+014	75.6 °C
H2	34ppa	33.58	0.333	1.71e+014	75.6 °C
H3	34ppa	33.34	0.333	9.85e+013	75.6 °C



Selected Detector: All
Well(s): A1-H12

721rust27-37



Detector = SYBR, Tm = 60.0 °C
Well(s): A1-H12

"Dissociation at same temperature" indicates presence of pathogen

721rust27-37

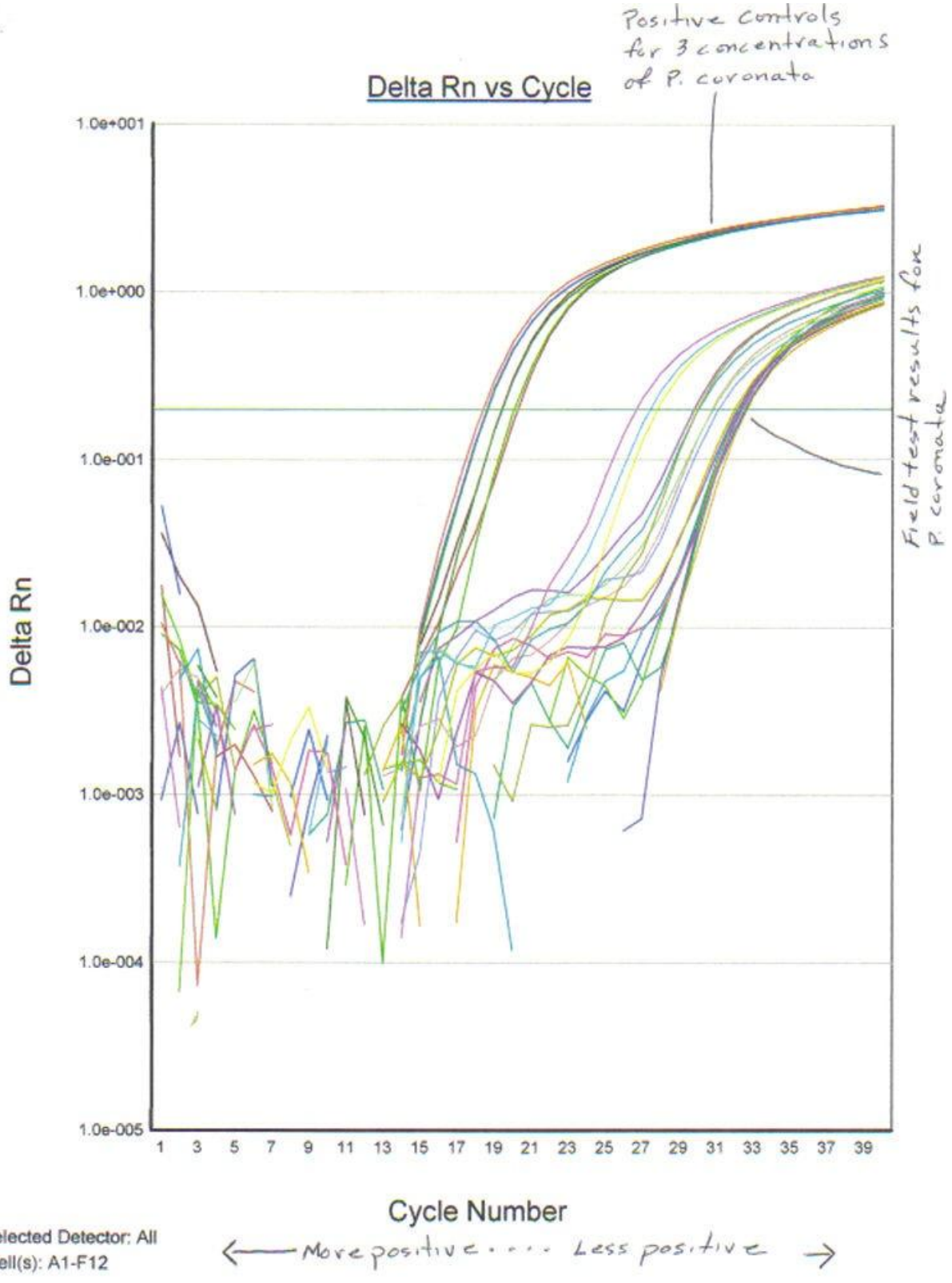
Page 3 of 3 (04/04/06 18:24:08

File:	2.08 06taqcoronata12-89
Print Date:	Tuesday, April 04, 2006 18:14:56
User:	Linda Kotolski
Plate Type:	Absolute Quantification
PCR Volume:	50 µL

Document Information

Operator:	Linda Kotolski
Run Date:	Wednesday, February 08, 2006 08:56:53
Last Modified:	Wednesday, February 08, 2006 11:45:35
Instrument Type:	Applied Biosystems 7300 Real-Time PCR System

Well	Sample Name	Ct	StdDev Ct	Qty
A4	10ng	20.21	0.113	10.00
A5	10ng	20.06	0.113	10.00
A7	20ng	19.32	0.009	20.00
A8	20ng	19.34	0.009	20.00
A10	40ng	18.32	0.127	40.00
A11	40ng	18.56	0.127	40.00
A12	40ng	18.52	0.127	40.00
C1	28	32.53	0.121	3.47e-004
C2	28	32.36	0.121	3.98e-004
C3	28	32.29	0.121	4.21e-004
C10	38	32.28	0.180	4.23e-004
C11	38	32.33	0.180	4.07e-004
C12	38	32.62	0.180	3.21e-004
D10	55	30.73	0.207	1.54e-003
D11	55	30.79	0.207	1.46e-003
D12	55	31.11	0.207	1.12e-003
E1	60	30.01	0.134	2.79e-003
E2	60	29.76	0.134	3.42e-003
E3	60	29.98	0.134	2.87e-003
E4	70	32.20	0.112	4.55e-004
E5	70	32.14	0.112	4.78e-004
E6	70	31.98	0.112	5.44e-004
F4	89	27.39	0.589	2.45e-002
F5	89	26.66	0.589	4.52e-002
F6	89	27.82	0.589	1.72e-002


Positive controls for 3 concentrations of P. coronata
Delta Rn vs Cycle
Field test results for P. coronata
1.0e+001
1.0e+000
1.0e-001
1.0e-002
1.0e-003
1.0e-004
1.0e-005
Delta Rn
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
Cycle Number
Selected Detector: All
Well(s): A1-F12
More positive .... Less positive
2.08.06taqcoronata12-89
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Addendum 2006

PEST PROS, INC.

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Mike Bandli
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2811 Agricultural Dr.
Madison, WI 53708-8911

September 20, 2006

Re: Final Report: Use of Polymerase Chain Reaction (PCR) and Spore Trapping for Efficient Fungicide Management of Asian Rust in Soybeans. Contract # 20008, 2006 extension.

A second season of rain water monitoring for Asian rust spores was implemented during the 2006 growing season. This effort utilized 8 independent crop consultants from through out the southern two thirds of the state and was expanded to include two sampling sites in Illinois, and one each in Iowa and Missouri. Trap filters were submitted for testing from late June into mid August. The summer was marked by generally dry conditions through out the mid west which limited the numbers of samples submitted. A total of 37 samples during the season were tested from Wisconsin sources. A sample from east central Wisconsin taken on 6/21 tested positive for Asian rust spores. Subsequent positives were recorded 7 times during the 2006 season in Wisconsin, and once in Missouri and confirmed by TAQMAN and electrophoresis gel (Table 1). No positives were recorded from the Illinois or Iowa samples. Three samples were submitted by the Missouri cooperator, two by the Illinois cooperator and the Iowa site submitted six filter samples. Cooperators were instructed to submit samples following a rain event exceeding 0.49 inches in 24 hours, or 0.75 inches in 2-3 consecutive days. CT values show that the spore load is likely at low levels.

Table 1: Asian Rust Samples Testing Positive

Location	Date	CT-Sybr	CT-Taq	Rainfall
Manitowoc, WI	6/21	32	35	0.55"
Plainfield, WI	6/24	35	34	0.50"
Manitowoc, WI	6/27	36	37	1.10"
Charleston, MO	7/15	36	38	na
Appleton, WI	7/24	36	37	0.8"
Appleton, WI	8/2	36	37	1.3"
Verona, WI	8/6	37	36	1.3"
West Bend, WI	8/6	35	36	0.73"
Sullivan, WI	8/6	37	36	0.65"

Each cooperator collected information on soybean crop development and health in fields adjacent to the trap. No cooperator could confirm the presence of Asian rust in their fields and no rust was reported by any party in their area. Rust development in the states of Texas and Louisiana where inoculum sources important to the Midwest must build. was minimal in 2006. Further the prevailing weather pattern in the midwest in 2006 was not considered conducive to significant northward movement of spores. Information on spore trapping activities conducted by USDA and other researchers will be presented at the up coming National Soybean Rust Symposium in St. Louis, MO. November 29- December 1 and compared to our results.

An SBIR grant proposal has been submitted to USDA to expand the trapping effort in 2007 to include five cooperators in the states of Minnesota, Iowa, Illinois, Michigan, Indiana and Wisconsin. The expanded effort will increase the chances that significant spore influxes associated with subsequent disease development can be documented , leading to the development of a predictive model of disease risk. This information will be integrated with aerobiological predictive models in development, to determine what form of predictive model best meets the needs of commercial soybean producers.

Randy Van Haren
Independent Crop Consultant